11.0 PRACTICAL CONSIDERATIONS

11.1 Test Method Transferability

Test method transferability addresses the ability of a method to be accurately and reliably performed by multiple laboratories (ICCVAM, 1997). This definition includes laboratories experienced in the particular type of procedure, and otherwise competent laboratories with less or no experience in the particular procedure. It also addresses whether the necessary facilities, equipment, and trained staff to perform the method can be readily obtained, and whether the cost of the assay and the level of expertise or training needed are considered reasonable. The degree of transferability of a test method affects its interlaboratory reproducibility.

The ICCVAM Submission Guidelines (ICCVAM, 1999) request a discussion of test method transferability with respect to the following factors:

- Availability of the facilities and the major fixed equipment needed to perform the test method;
- The training requirements for laboratory personnel to demonstrate proficiency with the test method;
- Costs involved in conducting the test; and
- Time needed to conduct the test.

11.1.1 Facilities and Major Fixed Equipment

The facilities needed to conduct ER binding assays are widely available, and the necessary equipment is readily available from major suppliers. Specific needs as related to the various *in vitro* ER binding procedures are described below. To ensure personnel and community safety, pertinent State or Federal regulations for the handling of hazardous and radioactive substances/wastes must be strictly adhered to.

Uterine Cytosol ER Binding Assays

Facilities: Standard toxicology, biochemistry, or molecular biology laboratory supplies, and an animal facility containing temperature, humidity, and light controls. A small animal surgical facility is recommended for laboratories that prefer not to purchase ovariectomized animals from animal suppliers.

Major Fixed Equipment: Refrigerated centrifuge, ultracentrifuge, and liquid scintillation counter.

MCF-7 Cells/Cytosolic Assays and Semi-Purified ERα and ERβ or GST Fusion Proteins

Facilities: Standard cellular or molecular biology laboratory with cell culture capabilities.

Major Fixed Equipment: Liquid scintillation counter.

Purified Human ER Measured by Fluorescent Polarization

Facilities: Standard cellular or molecular biology laboratory.

Major Fixed Equipment: Fluorescence polarization instrument.

11.2 Training Considerations

Uterine Cytosol ER Binding Assays

Basic laboratory skills and training in small animal handling and surgery.

MCF-7 Cells/Cytosolic Assays

Basic laboratory skills and training in cell culture techniques.

Semi-purified ERα and ERβ

Basic laboratory skills with training in molecular biology, particularly cloning, cell culture techniques and protein purification.

GST Fusion Proteins

Basic laboratory skills with training in molecular biology, particularly cloning, bacterial cell culture techniques and protein purification.

Purified Human ER Measured by Fluorescent Polarization

Basic laboratory skills.

11.3 Cost and Time Considerations

Table 11-1 provides information on the estimated cost per sample, the expected duration of the study, special equipment needed, and other considerations. The cost information provided was obtained from scientists working at not-for-profit institutions and would be an underestimate for studies conducted at contract laboratories in compliance with GLP guidelines. Where estimated costs are not provided, it is probably safe to assume that the costs for all of the uterine cytosol assays (RUC, MUC, RBA) are roughly equivalent. Similarly, it would be expected that the costs for the assays using semi-purified ER or GST constructs and the cell culture assays would be roughly equivalent.

Table 11-1 Comparison of Costs, Time, and Special Equipment Needs of Different ER Binding Assays

Assay	Cost/ Test substance	Duration (hours)	Special Equipment	Other Considerations
RUC	$135	~24 – 48	Liquid scintillation counter ($15K - $30K)	
MUC	n.a.	~24 – 48	Liquid scintillation counter ($15K - $30K)	
RBC	n.a.	~24 – 48	Liquid scintillation counter ($15K - $30K)	
MCF-7 cytosol	n.a.	~24 – 48	Liquid scintillation counter ($15K - $30K)	
hERα	n.a.	~24	Liquid scintillation counter ($15K - $30K)	
hERα-FP	$65	~4	Fluorescence polarimeter ($20K - $35K)	No radioactive wastes. Proprietary fluorescein-labeled estrogen ligand.
hERβ	n.a.	~24	Liquid scintillation counter ($15K - $30K)	
rERβ	n.a.	~24	Liquid scintillation counter ($15K - $30K)	
GST-hERαdef	$30	~8	Liquid scintillation counter ($15K - $30K)	
GST-mERαdef	$30	~8	Liquid scintillation counter ($15K - $30K)	
GST-aERdef	$30	~8	Liquid scintillation counter ($15K - $30K)	
GST-cERdef	$30	~8	Liquid scintillation counter ($15K - $30K)	
GST-rtERdef	$30	~8	Liquid scintillation counter ($15K - $30K)	
MCF-7 cells	n.a.	~24 – 48	Liquid scintillation counter ($15K - $30K)	

n.a. = Cost estimate not available in the literature or from laboratories conducting the assay.